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Germline *WT1* Mutations in Wilms' Tumor Patients: Preliminary Results

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We conducted a comparative study of the prevalence of germline *WT1* mutations in patients with Wilms' tumor. Patients in Group 1 have familial Wilms' tumor, bilateral disease, associated urogenital anomalies, and/or second cancers. Those in Group 2 are unilateral, sporadic Wilms' patients without other associated conditions. Patients with aniridia or Denys-Drash syndrome are known to have *WT1* alterations, and are excluded from this study. Preliminary results on 96 subjects show that the overall germline *WT1* mutation frequency is low (<5%). The work to date establishes the feasibility of identifying patients with germline *WT1* mutations and, in the future, offering genetic predisposition testing to at-risk relatives. However, genetic predisposition testing of children for *WT1* mutations raises many ethical, legal, and psychosocial issues; research is needed to evaluate risks and benefits.

Key words: germline *WT1* mutations, Denys-Drash syndrome, genetic predisposition

INTRODUCTION

Approximately 400 new cases of Wilms' tumor occur annually in the United States [1]. The incidence of the tumor is 8 per million per year in children under 15 years of age [2]. Despite its rarity, Wilms' tumor is a paradigm of genetic heterogeneity of inherited cancer susceptibility, i.e., germline mutations in one of several genes can predispose to development of the tumor [3–5].

In 1964, Miller et al. [6] showed that Wilms' tumor patients have a high frequency of certain birth defects. Congenital aniridia (absence of the iris) is found in approximately 1% of Wilms' tumor patients, a 1,000-fold higher than its rate in the general population [7]. Some patients with the *W*ilms' tumor-*a*niridia association also have *g*enitourinary (GU) malformations and mental *r*etardation, which constitute the WAGR syndrome [3,8]. Part of this constellation of defects overlaps another Wilms' tumor-associated syndrome, Denys-Drash syndrome, which features Wilms' tumor, pseudohermaphroditism, hypertension, and renal failure [9]. Subsequent studies have shown that the concurrence of aniridia and Wilms' tumor is attributable to germline deletions of chromosome 11p13, wherein genes for aniridia and Wilms' tumor are located and therefore constitute a contiguous gene syndrome [3,10]. In 1990, this Wilms' tumor gene, *WT1*, was identified and found to contain four zinc-finger domains that characterize regulators of DNA transcription [11,12].

Cloning of the *WT1* gene prompted us to study the frequency of germline *WT1* mutations among defined subgroups of Wilms' tumor cases.

MATERIALS AND METHODS

We initiated a comparative study of the frequency of germline *WT1* mutations in several subsets of Wilms' tumor patients. Patients in Group 1 have Wilms' tumor showing one or more of the following features that might indicate inborn susceptibility: bilaterality, family history of Wilms' tumor, associated GU anomalies, and/or later occurrence of a second cancer [13]. Intralobar nephrogenic rests are considered a urogenital defect in this study based on reports of its association with the *WT1* locus [8,14]. Patients in Group 2 have sporadic (nonfamilial), unilateral Wilms' tumor without GU anomalies and second cancers; their disease is thought to arise primarily from somatic mutations [15,16]. Excluded from the study are Wilms' cases with associated aniridia or Denys-Drash syndrome, who are known to have inherited *WT1* mutations [3]. Also excluded are patients with Beckwith-Wiedemann syndrome, which is associated with a second Wilms' tumor locus on chromosome 11p15 [3,17].

The study was designed to accrue 100 subjects in

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Group 1 and 100 subjects to Group 2, including substantial numbers of patients with uncommon clinical features. Ascertainment was initially attempted through a cooperative treatment network, the National Wilms' Tumor Study (NWTS), which has collected demographic and epidemiological data on each enrollee through participating institutions [18,19]. We obtained lists of living patients from the NWTS files who qualified as a member of either group. The principal investigators at member institutions with eligible patients were contacted for permission to approach the subjects. With signed consent from both the physician and parents of the Wilms' patient, a blood specimen was obtained for *WT1* analyses.

Blood specimens were coded, and the laboratory was blinded with regard to the personal and family history of the subjects. DNA was extracted according to a standard protocol [20]. Analysis of the *WT1* gene included Southern blotting for large deletions, polymerase chain reactions (PCR) to amplify each of the 10 exons of the *WT1* gene, single-strand conformational polymorphism (SSCP) analysis for abnormal mobility on gel electrophoresis, and direct sequencing to detect and characterize alterations [21]. SSCP analysis of each exon is performed twice, once without glycerol and once with 10% glycerol [22]. When a mutation is found, the defect is confirmed by repeating the entire laboratory procedure: extraction of DNA from a new aliquot of stored blood and repeating the PCR amplification, SSCP analyses, and DNA sequencing [21].

The study is still in progress; these interim results were reported at the 1995 Conference on Renal Cancers in Children. To speed completion of the study, additional samples have also been obtained using the same eligibility criteria from the Pediatric Oncology Group (POG) Wilms' tumor bank and the Dana-Farber Cancer Institute.

RESULTS

We have completed analyses for germline *WT1* mutations in 96 Wilms' patients to date. Four germline alterations were identified among these 96 patients (4.2%) (Table I). All four alterations were found among the 74 subjects of Group 1 (5.5%), and none among the 22 subjects of Group 2 ($P > .05$). The spectrum of changes include splice site alteration (one patient), nonsense mutations (two), and deletion (one). An additional 104 blood specimens of Wilms' patients have been collected and are undergoing study to reach the goal of analyzing 100 subjects in Group 1 and 100 subjects in Group 2.

The low frequency of germline *WT1* mutations among the subjects of Group 1 is unexpected. After excluding patients with aniridia or Denys-Drash syndrome, germline *WT1* mutations are present in only a small fraction of Wilms' tumor patients with familial disease, bilateral tumors, second cancers, or associated GU anomalies; alternative explanations are needed to explain these clinical associations.

TABLE I. Germline *WT1* Alterations in 96 Wilms' Patients

Wilms' patient category	No. with *WT1* alterations
Group 1	4/74
Familial	0/4
Bilateral	0/2
GU anomalies, including rests[a]	2/47
Second cancer	0/5
Multiple factors[b]	2/16
Group 2[c]	0/22
All Patients	4/96

[a]Intralobar nephrogenic rests only.
[b]More than one of the above features.
[c]Unilateral, sporadic Wilms' tumor with no GU anomalies.

DISCUSSION

The molecular basis for the association of Wilms' tumor with GU anomalies might involve the pleiotropic expression of the *WT1* gene [4,8,23]. *WT1* is expressed in several tissue components of the fetal kidney, as well as the nongerm cell components of the testes and ovaries of humans and other species [23–25]. The occurrence of the WAGR syndrome indicates that a germline *WT1* deletion might be sufficient to produce urogenital anomalies and, additionally, predispose to the development of Wilms' tumor [3]. However, Wilms' tumor development requires additional mutations, including inactivation of the second *WT1* allele [26]. Additionally, nearly all Denys-Drash patients have germline *WT1* mutations within exon 9 that cluster at or near codon 394 [22,25]. This narrow *WT1* mutational spectrum suggests a tight genotype-phenotype relationship. Surprisingly, the frequency of *WT1* mutations is low among Wilms' tumor with only GU anomalies, and the biological basis of this association remains to be fully explained [27]. The findings of our interim report are based on small numbers, however, and additional data may alter these findings.

WT1 is one of at least three candidate inherited susceptibility genes for Wilms' tumor [3]. A second Wilms' tumor locus, *WT2*, was identified in Wilms' tumor cases with a second constellation of malformations featured in Beckwith-Wiedemann syndrome: macroglossia, omphalocele, visceral cytomegaly, and partial or complete hemihypertrophy. Gene mapping studies have localized the Beckwith-Wiedemann locus to chromosome 11p15 [17]. A third locus was recognized through studies of familial Wilms' tumor, which is found in approximately 1% of Wilms' tumor cases. Linkage studies of four large families with Wilms' tumor have excluded *WT1* and *WT2* as the inherited defect in these families [28]. Genetic heterogeneity of familial Wilms' tumor has been shown by our report of a patient who had unilateral Wilms' tumor, GU

anomalies, and a germline *WT1* mutation inherited from his father who had a unilateral Wilms' tumor but no malformations [29]. As additional susceptibility genes are identified, our repository of specimens can provide estimates of germline mutation frequencies.

While our study was in progress, other investigators reported that somatic mutations in the *WT1* gene of Wilms' tumor cells are infrequent (less than 10%) [30,31]. The low frequency of somatic *WT1* mutations is consistent with our finding of infrequent germline mutations in the gene. Other studies suggest that *WT2* germline and somatic mutations may be more common in Wilms' tumor patients [3,32].

Recently, public and professional concerns have been raised about ethical and consent processes for genetic studies, particularly in children [33–35]. In this study, confidentiality has been ensured by the removal of names before specimens were sent for laboratory analyses [36]. Information linking specimens to patients in computer and paper files is locked, and access restricted to a few members of the research group. In addition to approval from the Principal Investigator's Institutional Review Board (IRB), approval was obtained from IRBs of other hospitals that enrolled eligible patients, if required. The review process became more complex and lengthy as media attention to genetic testing increased.

Consensus appears to be developing on the need to disclose the results of genetic studies, particularly when the data are clinically useful [33]. However, disclosure is a complex process that requires patient education, counseling, psychological support, and follow-up medical care [37,38]. The responsibilities for providing these services and their associated costs have not been clearly defined. The consequences of genetic information are often lifelong and profound, particularly when study subjects are children [36]. Genetic results may substantially alter a child's self-image and weaken bonds between parent and child [39]. The susceptible child might be stigmatized and isolated, or develop the "vulnerable child syndrome" in which parents become overly protective due to feelings of guilt. In addition, ages and level of maturity required for assent or consent to participate in genetic testing and disclosure are uncertain. Educational and counseling services need to account for the age of the subject, ages at risk of cancer, the lethality of the tumor, and opportunities for early interventions [34].

In the emerging area of genetic predisposition testing for cancer susceptibility, the main focus has been adult-onset carcinomas of the breast, colon, kidney, and other sites [38]. In our *p53* genetic predisposition testing program for families with Li-Fraumeni syndrome, eligibility is presently limited to adults because of concerns about safety and confidentiality. However, genetic predisposition testing of families with adenomatous polyposis coli *(APC)* for germline *APC* mutations has included children. The difference is explained by the availability of early interventions for *APC* carriers [40]. In contrast, *p53* carriers develop cancer in many organ sites, and early detection procedures are costly and might not result in earlier diagnosis and improved survival [34].

The usual screening modality for Wilms' tumor is periodic abdominal ultrasound to detect renal masses [3,7]. There is general agreement that young children with aniridia, Beckwith-Wiedemann syndrome, or a history of familial Wilms' tumor should be screened for the neoplasm [3]. High-risk infants who are relatives of *WT1* carriers should be added to the list of candidates for periodic screening, even though penetrance appears low [41]. The issue of screening young relatives of Wilms' cases with no detectable *WT1* mutation is more problematical. Siblings might still be at risk from a germline mutation in a gene other than *WT1* [5]. Confidence regarding the interpretation of negative results should increase when all predisposing genes have been identified.

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COMMENTARY

In this preliminary report, Li et al. demonstrate the feasibility of identifying patients with germline *WT1* mutations. However, among the issues that need to be resolved for this particular cancer is the identification of additional genes involved in the development of Wilms' tumor: *WT1* is only one of at least three genes involved in its tumorigenesis. Also, the authors remind us of the fact that the routine use of genetic predisposition testing for at-risk individuals raises many ethical, legal, and psychosocial issues which need to be addressed.